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This research assesses whether exposing birch bark to methanol or ethanol vapours as used in treatments to reshape distortions in bark artifacts causes colour changes. Analysis of water, methanol and ethanol extracts of bark by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) confirmed that methanol and ethanol solvents extract similar compounds in similar relative abundances from birch bark, while water presents a different extraction profile. Three barks of different colours on their cambium sides were exposed to methanol or ethanol vapours for two different exposure periods, and were monitored visually as well as through colour measurements and weight gain for up to 35 weeks after treatment. The short (2.3-day) methanol vapour exposure gave the best results: low colour change combined with fast absorption rate and sufficient peak weight gain to improve bark flexibility. Analysis was carried out on samples of a white crystalline deposit that occurred on one of the barks exposed to ethanol vapour: it was identified as betulin and lupeol in approximately a 2:1 ratio. Cleaning the vapour-exposed barks with swabs moistened with water was successful in removing the white residue and did not in itself cause colour change.

Cette recherche évalue le risque de changement de couleur de l'écorce de bouleau au cours de traitements de remise en forme à l'aide de vapeurs d'éthanol ou de méthanol. L'analyse d'extractions d'écorce dans de l'eau, du méthanol et de l'éthanol obtenue par pyrolyse-chromatographie en phase gazeuse-spectrométrie de masse a confirmé que comme solvants, le méthanol et l'éthanol extraient de l'écorce des composés semblables et dans les mêmes quantités relatives. L'eau, par contre, présente un profil d'extraction différent. On exposa trois échantillons d'écorce qui était de couleur différente sur leur côté du cambium à des vapeurs de méthanol ou d'éthanol et ce, pendant deux temps d'exposition distincts. Pendant ces expériences et jusqu'à 35 semaines après traitement, on procéda régulièrement à l'évaluation visuelle et aux mesures colorimétriques des deux surfaces des écorces et on mesura leur gain en poids. L'exposition la plus courte (2,3 jours) aux vapeurs de méthanol a donné les meilleurs résultats : peu de changement de couleur tout en ayant un taux rapide d'absorption des vapeurs de solvant et une augmentation de poids suffisamment élevée pour que la flexibilité de l'écorce soit améliorée. Un dépôt blanc crystallin qui apparut sur un des échantillons d'écorce exposé aux vapeurs d'éthanol fut analysé et identifié comme étant un mélange de bétuline et de lupéol dans des proportions 2:1. Le nettoyage de la surface des écorces à l'aide de coton-tiges humectés d'eau ne cause aucun changement de couleur et réussit, le cas échéant, à enlever ces dépôts crystallins.

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INTRODUCTION

Methanol and ethanol vapours are used to temporarily soften birch bark for the purpose of reshaping bowed, curled or distorted artifacts.[1-4] In 1986, Gilberg recommended these for plasticizing birch bark following his investigation of eight solvent vapours.[2] He concluded that methanol or ethanol vapours were effective at relaxing birch bark without "significant" negative effects such as delamination or discoloration. Building on Gilberg's work, the authors recently published a study that assessed and compared more closely the effectiveness of methanol and ethanol vapours in softening and reshaping birch bark, and that examined whether modifying treatment parameters such as vapour exposure time could improve the treatment outcome.[5] This present study aims to quantify and compare the degree to which methanol and ethanol vapour treatments cause colour changes to birch bark using two of the same sample sets as the previous study. A parallel study by the authors investigated colour changes (fading and discoloration) due to exposure to light on some of the same bark sample sets.[6]

Birch Bark Structure, Composition and Properties

The outer bark of the white birch tree (*Betula papyrifera* Marsh.), also called the paper birch, is commonly used by the Indigenous peoples of North America to create objects such as containers, canoes, cradles and decorative bitten bark patterns. These objects are frequently found in museum collections around the world.

As a material, birch bark is composed of layers of closely packed cork cells: thin-walled cells that contain relatively more betulin and other resinous materials alternate with thicker-walled cells that are dark and contain relatively more tannins and other phenolics.[2,7] This laminated structure results in naturally peeling sheets.

Birch bark's main constituent by weight is suberin (30%–50%),[8] a crosslinked biopolyester formed predominantly from long-chain ω-hydroxyacids and α,ω-dicarboxylic acids. It can make up nearly 85% of cork cell walls.[2] It is a hydrophobic compound that gives the birch bark protective barrier qualities against water,[8] as well as its characteristic flexibility.[2] Recent analysis of the outer bark from a related species *Betula pendula* (silver birch) identified the distribution of components as 44% w/w suberin, 40% w/w extractives such as betulin and phenolics, 9% w/w lignin and 4.5% w/w polysaccharides.[9] The low lignin and very low cellulose content (1.8% by weight, a polysaccharide) distinguish birch bark from wood.[7] Tannins are also found in relatively small amounts: in white (paper) birch they range from 1.6 to 3.3%.[10]

The innermost side of birch bark is called the *cambium side* (the side closest to the tree's bark cambium layer), while the outer layer (on the tree's exterior surface) is called the *paper side*. Depending on factors such as the time of year it is harvested, the colour of the cambium side can range from light (e.g., yellow or beige) to dark (e.g., orange, red or brown).[11] The white and grey birch species have a characteristic white bark exterior surface (the paper side). A white powder easily rubs off of this surface. This powder contains betulin (a white crystalline triterpene) and other lupane triterpenes, characteristic major components of birch bark.[10] Pentacyclic triterpenes including betulin can be found in birch bark in the range of 20–45% by weight.[8] Betulin is known to have antiseptic properties and is associated with birch bark's natural resistance to mould and insect attacks.[2]

Birch bark sheets naturally curl with their paper sides inwards. Taking advantage of this natural curvature, many curved or rounded objects (containers, canoes) are constructed with the cambium side outwards. Although the suberized cork cells are very flexible when first harvested from the tree, birch bark stiffens as it ages and takes on a permanent set.[2] When used later for forming and assembly into objects, birch bark sheets may be soaked in water to regain flexibility,[12] sometimes with heat from a fire.[13]

Birch Bark Damage and Treatments

Birch bark objects can be damaged by physical forces such as poor handling and poor storage, or, if the bark is assembled with or fixed to another type of material (e.g., wood, root lashings), by stresses resulting from differential responses to environmental changes. Such damage may cause the birch bark to spring out of shape or curl. Aged birch bark is usually too stiff to move back into its original shape without risk of cracking or splitting.

Birch bark's hydrophobic nature means that unlike many other organic materials, it does not respond well to humidification treatments aimed at making it pliable enough to allow reshaping. Steam can work to this effect, but the heat is the main reason the birch bark softens, rather than the water.[2] There are several disadvantages in using steam: most often only localized softening is obtained, which leads to stresses during reshaping and may result in cracking and splitting; steam's softening effect dissipates quickly thus the working time window for reshaping is quite short, which may also lead to the occurrence of stresses; and there are high risks of condensation leading to tidelines or staining.[2,3,14] Although dimethylformamide, acetone and ethyl cellosolve (2-ethoxy ethanol) vapours were found by Gilberg[2] to readily plasticize birch bark, they also caused considerable discoloration and delamination. By comparison, ethanol vapours and, even more effectively, methanol vapours were found to successfully plasticize birch bark with little associated delamination or discoloration. Gilberg concluded by recommending the use of methanol or ethanol vapours. A more recent study by the authors expanding on the Gilberg study found that methanol vapour was more effective in softening and reshaping birch bark than ethanol vapour, but that the latter can be effective provided longer vapour exposures are used.[3]

Colour Changes Due to Solvents

It is known that contact with solvents can cause birch bark to change colour. Agrawal and Bhatia mentioned the risk of colour change from "use of any solution" in stain removal treatments on birch bark manuscripts from India (probably bark from the Himalayan species *Betula utilis*).[15] Subsequently, Agrawal et al. reported more specifically that an Indian species of birch bark cleaned (probably by immersion) with various organic solvents (ether, acetone, benzene and toluene) produced "a white appearance" and explained: "Possibly this is because of the solubility of a portion of birch-bark in the solvent."[16] They also found that methanol produced "slight white spots"; ethanol, a "whitish colour reversion [*sic*]"; and cold or hot water, no change.[16] A further study by Agrawal and Suryawanshi found that immersion in ethanol for "at least 2 hours" caused some separation of thin layers of birch bark and a "little change" in appearance, with the bark becoming less bright (gloss reflectance meter measurements went from 46% to 39%).[17] Later, Suryawanshi reported that ethanol immersion extracted approximately 10 wt% of birch bark constituents.[18] Yamauchi found that after a 24-hour immersion of birch bark in ethanol, material was extracted which upon solvent evaporation formed a white crystalline compound that was identified as "one of the constituents of birch bark such as betuline [*sic*]."[19] The bark samples had correspondingly shrunk slightly in size and darkened, the paper side becoming light yellowish-brown and the cambium side, dark yellowish-brown. Yamauchi also mentioned that bark "quickly dipped" in a 1:1 water:ethanol bath did not produce shrinkage or any colour change.[19] Klügl noted that with prolonged (e.g., 15 months) immersion in ethanol or other polar non-aqueous solvents, betulin "disappears" from the bark and the bark colour changes from white to brown, the darkening caused "mostly by the removal of betulin and to a small extent probably by oxidation reactions."[7] Anastassiades found that using 1:1 ethanol:water on swabs to clean adhesive tape residue from the cambium side of birch bark (*Betula papyrifera* Marsh.) "changed the colour of exfoliating areas of bark from a brownish-white to reddish-pink" but using a very small amount of ethanol on a crepe block caused no observable colour change.[20]

Colour Changes Due to Solvent Vapour Exposures

The effects of solvent vapours are less well known than the effects of solvents. In their case study using solvent vapours to plasticize and unroll a birch bark scroll, Gilberg and Grant exposed the bark to methanol vapour for 2–3 weeks and reported no visible discoloration of the bark surface.[1] Gilberg reported that dimethylformamide, acetone and ethyl cellosolve (2-ethoxy ethanol) vapours caused considerable discoloration, while better results – plasticization with little associated discoloration – were obtained with methanol vapour and to a lesser extent with ethanol vapour.[2] Gilberg noted that the discoloration caused by methanol or ethanol was primarily confined to the paper side, where only the loose, exfoliated, papery layers yellowed after prolonged exposure; the cambium side showed little to no change, albeit sometimes a slight reddening. Gilberg also found that discoloration with these two solvent vapours appeared more pronounced with

relatively new birch bark. Finally, he described a white bloom appearing on the surface of some samples exposed to methanol or ethanol vapour, which he attributed to the dissolution and deposition of betulin at the surface of the bark. Gilberg stated that the appearance of bloom could be minimized by covering the bark during exposure with a sheet of polyester (Melinex) to slow down the solvent evaporation rate.[2] Maitland captured, under magnification, timed images of a small area of the paper side of a Kashmiri birch bark manuscript exposed to methanol or ethanol vapours over the course of 12 minutes, which showed a slightly higher redeposition of white surface efflorescence on the ethanol-exposed area (reported as likely a triterpene).[4]

Research Objectives

In this study, the degree to which exposure to methanol or ethanol vapour causes colour changes was investigated in three stages. Firstly, methanol and ethanol extracts from different barks were analyzed to ascertain which bark compounds could be solubilized by immersion in methanol or ethanol, as these same compounds may also be at risk of migrating to the bark surface during solvent vapour exposure and affect its colour. Secondly, solvent vapour exposure experiments were carried out on samples of three barks differing in colour on their cambium side. The barks were exposed to methanol or to ethanol vapour for two exposure periods, and their colour before treatment (BT) and after treatment (AT) was assessed visually and with colorimetric measurements. Sample weights were monitored before, during and after vapour exposures in order to measure the barks' relative solvent vapour absorption and desorption. Surface deposits that appeared on some samples following solvent exposure were analyzed and identified. Finally, surface cleaning was carried out using swabs moistened with water to determine whether any deposits that may have appeared on the surface due to vapour exposure could be removed and whether such cleaning would result in additional colour changes.

EXPERIMENTAL

Birch Bark Samples

Large sheets of outer bark from six different white birch (*Betula papyrifera* Marsh.) trees were harvested in 2012–13 in Maniwaki, Quebec by Daniel Smith, an Anishnabeg canoe craftsman, and stored indoors until transfer to CCI in 2014. The sheets bowed naturally, forming large cylindrical segments with the white paper (exterior) side curved inwards. A range of colours on their cambium side was apparent (**Figure 1**). The dark red bark A is most likely *winter bark*, harvested late in the year or early spring and known to be dark in comparison to the paler tones of *summer bark* harvested in early summer while the tree is growing.[11]

Barks that represented the range of cambium side colours and provided sufficient quantity to make up all required samples were selected for each experiment. Small samples of all six barks (A to F) were immersed in methanol, ethanol, acetone and water for solvent extraction. Dried extracts from barks which exhibited the widest range of colour – Bark A (dark red), Bark C (reddish orange) and Bark E (beige) – were analyzed by Py-GC-MS. Three barks were selected for each of the two vapour exposure experiments. Bark B (brown), Bark C (reddish orange) and Bark E (beige) were selected for Experiment 1: visual assessment and colorimetric measurements after two different exposure periods to methanol or ethanol vapour, as well as after swab cleaning with water. The paper side was assessed only for Barks C and E because Bark B was too inhomogeneous to allow visual and colorimetric assessments. For Experiment 2, which further investigated the appearance and cleaning of the white bloom noted on Bark C during Experiment 1, Bark A (dark red), Bark C (reddish orange) and Bark E (beige) were selected. Characteristics of Barks A, B, C and E used in Experiments 1 and 2 are described in **Table I**. Control samples for each bark type were kept in the CCI climate-controlled laboratory environment, shielded from light in an acid-free cardboard box.

Solvent Extractions

For the extractions, birch bark pieces were immersed in solvent in sealed glass vials at a ratio of 1 g of bark to 25 mL solvent and extracted for seven days. The solvents tested were methanol, ethanol and water; an acetone extraction was also carried out for comparative purposes. The solutions were uncovered and left to air dry in a fume hood. All extracts were assessed visually and photographed.

Identification of Chemical Compounds by Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

Sampling

A subset of extracts was chosen for analysis by Py-GC-MS. Samples of the dried extracts of Barks A, C and E were selected to compare the composition of extracts from different

Figure 1. Samples of six birch barks showing a range of colours on the cambium side. Samples of the six barks were solvent extracted (see Figure 2). Samples B, C and E (solid red circles) were used in Experiment 1 (visual assessment and colorimetric measurement after vapour exposure), while samples from Barks A, C and E (dotted circles) were used in Experiment 2 (further visual assessment following vapour exposures) and their extracts were analyzed by Py-GC-MS.

Table I. Characteristics of birch bark samples tested in Experiments 1 and 2.

	Bark A	Bark B	Bark C	Bark E
Colour (see Figure 1)	dark red	brown	reddish orange	beige
Date harvested	2012–13	2012–13	2012–13	2012–13
Ave. thickness (mm)*± std. dev.	3.0±0.2	1.7±0.2	2.6±0.6	1.8±0.1
Ave. density (g/cm^2)±std. dev.	0.56±0.1	0.55±0.05	0.54±0.06	0.52±0.01
BT Weights (g, ±0.01 g)				
Sample Me-2d	Not measured	7.88	9.90	3.91
Sample Me-1w		12.70	13.51	17.48
Sample Et-1w		10.77	16.18	6.72
Sample Et-3w		9.35	15.00	13.22

*Average based on 5 samples × 6 micrometer measurements each.

barks using the same organic solvent, and of the same bark using different solvents (methanol, ethanol and water). Samples were first scraped from the vials and finely ground in their entireties. In addition, areas of whitish and orange residue were separately sampled from the Bark E-methanol extract. Approximately 50 µg were used for each Py-GC-MS analysis.

As well, approximately 10 µg of the surface deposit produced during Experiment 2 on a sample of Bark C exposed to ethanol vapour was sampled using a scalpel and analyzed by Py-GC-MS.

Py-GC-MS

Surface residue and bark extract samples were placed into micro-vials (Agilent Technologies, part no. 5190-3187) with 1.8 µL of tetramethylammonium hydroxide (TMAH, Supelco, Bellafonte, PA) in methanol (1:25). The micro-vial was inserted into a thermal separation probe (TSP, Agilent Technologies, Inc., Palo Alto, CA) installed in a multimode inlet on an Agilent 7890A GC interfaced to a 5975C MS. The multimode injector with TSP was operated in split mode (8:1 split for the surface residue and 40:1 split for the bark extract residues) ramped from 50°C to 450°C at a rate of 900°C/minute to perform the pyrolysis. The final temperature was held constant for three minutes and then decreased to 250°C, at a rate of 50°C/minute, for the remainder of the run. For the GC separation, a Zebron ZB-5MSi fused silica column (30 m x 0.25 mm i.d., 0.25 µm film thickness; Phenomenex Inc., part no. 7HG-G018-11) was used. Ultra-high purity helium carrier gas (99.999%) was used with a constant flow of 1.2 mL/minute. The oven was programmed from 40°C to 200°C at 10°C/minute and 200°C to 310°C at 6°C/minute with a hold time of 20 minutes (54.33 minutes run time).The MS was operated in EI positive mode (70 eV). The MS transfer line temperature was 280°C; the MS ion source was held at 230°C and the MS quadrupole at 150°C. The MS was run in scan mode from 50–550 amu (5–25 minutes), 50–600 amu (25–30 minutes) and 50–650 amu (35–54.33 minutes). Data were processed using Agilent ChemStation software, v.E.02.02. Mass spectral identification was also performed using the NIST 11 Mass Spectral Library, published data[21,22] and the analysis of birch bark reference material.

Vapour Exposure Treatments

Exposures to methanol and ethanol vapour were carried out following the procedure used in previous research[5]: after cleaning the samples on both sides using a soft brush and vacuum cleaner, the bark samples were placed cambium side down on a corrugated plastic (Corex) sheet and sealed inside doubled polyethylene bags (one inside another) with four open jars of either methanol or ethanol (95% anhydrous). As the samples absorbed solvent vapour and softened over the course of the exposure period, cotton-covered sandbags weighing up to 1 kg per sample were applied as necessary to counteract the barks' natural tendency to curl and keep the barks relatively flat. Note that the cotton-covered sandbag weights may have affected the barks' rates of vapour absorption at this stage.

Experiment 1 was carried out for visual assessment and colorimetric measurements on Barks B, C and E after two vapour exposure periods for each solvent and after subsequent swab cleaning. Since it was known from previous CCI treatments that methanol vapour can soften birch bark within a few days, and since recent research[5] showed that ethanol vapour absorbs more slowly than methanol vapour, it was decided to test and compare longer ethanol vapour exposures with respect to the methanol exposures, as follows:

- Methanol (Me): short exposure = 2.3 days (Me-2d) and long exposure = 1 week (Me-1w)
- Ethanol (Et): short exposure = 1 week (Et-1w) and long exposure = 3 weeks (Et-3w)

Three samples, one from each bark (B, C and E), were placed together in the same sealed double bag and exposed to the same solvent vapour for the same period. After solvent exposure, the bark samples were dried between a double layer of cotton rag blotting paper and flat melamine boards under weights totaling 1.5–1.8 kg/sample (enough to flatten the samples; the load varied across bark sets but was consistent

within a set) for 1 week, with blotters changed every day, after which the samples were left to air out unrestrained.

Experiment 2 was carried out to further investigate the occurrence and cleaning of the white bloom observed on Bark C in Experiment 1. Two samples of each bark (A, C and E) were placed in their own sealed double bags with either methanol or ethanol solvent jars and exposed for a period of 1 week. After exposure, the bark samples were dried in between double blotters and under flat melamine boards and weights as in Experiment 1, for either 1 week (T1) or 8 weeks (T2). Blotters were changed every day the first week, then once a week thereafter for the T2 samples. After the flattening periods T1 or T2, samples were left to air out unrestrained.

Visual Assessments

Colour was assessed visually using photographs of samples taken at specific points in each experiment. In Experiment 1, photographs of Barks B, C and E before treatment (BT) were compared to photographs taken after vapour exposure and air drying (after 35 weeks of airing out except for Me-2d samples which aired out for 33 weeks) and to photographs of swab-cleaned samples taken 2 weeks later (after 37 weeks of airing out, 35 weeks for Me-2d samples). In Experiment 2, the after treatment (AT) photographs used for visual comparison with the BT photographs were taken after 39 weeks of airing out for Bark A, and after 21 weeks for Barks C and E.

Colorimetric Measurements

A Minolta CM-2600D hand-held spectrophotometer (diffused illumination and 8° viewing (d/8)) was used to measure bark colour throughout Experiment 1, at the following times:

- BT: before treatment
- AT-1d: after the vapour exposure periods + 1 day of drying between blotters under weights
- AT-1w: after the vapour exposure periods + 1 week of drying between blotters under weights
- AT-8w: after vapour exposure + 8 weeks of drying (1 week as above + 7 weeks air drying unrestrained)
- AT-35w: after vapour exposure + 35 weeks of drying as above (33 weeks for Me-2d days samples); this set is the "AT-35 weeks Before Cleaning"
- AT-37w: 5 days after swab cleaning (see description below) which was done 8 to 9 days following air drying; this set is the "AT-37 weeks After Cleaning"

Samples were kept covered with tissue paper or kept in an acid-free cardboard box to avoid being exposed to any appreciable amount of light that would have contributed to colour change.[6] Barks C and E were monitored on both their cambium and their paper sides, while Bark B was monitored only on its cambium side because its paper side was too inhomogeneous.

Six spots were measured on each sample. The reading for each spot was an average of three measurements. Areas of bark with lenticels (elongated pores in the bark that allow gas exchange between the atmosphere and the internal tissues), knots, residues or previously extant stains were avoided. For Bark E, only the smooth beige areas of the cambium layer were assessed and not the darker brown areas which were residues from the rougher inner bark. The measurements of the six spots were averaged and graphed. The average colour change CIEDeltaE2000 (ΔE2000) and changes in lightness (L*), red-green (a*) and yellow-blue (b*) for each sample were plotted against time (with T = 0 corresponding to end of solvent exposure and beginning of drying). Curve fitting of the data was done using Microsoft Excel, SigmaPlot and Simfit in order to show the general trend of the data. The standard deviation is the variation of the six spots.

Weight Gain

To track solvent absorption and desorption in Experiment 1, bark samples were weighed before, during and after solvent vapour exposure using a Mettler PC 4400 scale, accurate to ± 0.02 g. Samples were weighed at the following intervals: before treatment and after vapour exposure plus 1 day, 1 week, 8 weeks, 16 weeks and 41.3 weeks (289 days) of airing out. Samples exposed to ethanol vapour for 3 weeks were also weighed once each week during the exposure treatment.

Weights of the AT bark samples relative to their BT weight were calculated (ATweight/BTweight, expressed as a %) at the end of solvent vapour exposures (at T = 0), just before the start of airing out. Thus, a value of 100% means that the AT weight was the same as the BT weight and the value above 100% is the weight *gain*. If Z is the relative weight (ATweight/BTweight), the error ΔZ was calculated based on the formula:

$$\Delta Z/Z = \Delta ATweight/ATweight + \Delta BTweight/BTweight$$

$$\Delta Z = (\Delta ATweight/ATweight + \Delta BTweight/BTweight) \times (ATweight/BTweight)$$

The ΔBTweight and ΔATweight were each estimated at 0.05 g to account for the accuracy of the scale (± 0.02 g) and small variabilities in weights due to changes in RH.

Swab Cleaning Tests

Following the vapour exposures of Experiment 1, swab cleaning was carried out in a consistent manner on all samples (Barks B, C and E) to assess colour change due to both the removal of any surface deposits present and the application of a small amount of water. Cleaning was carried out after solvent vapour exposures and 36 weeks of airing out for the barks exposed to Me-1w, Et-1w and Et-3w, and after 34 weeks for the barks exposed to Me-2d. Cotton swabs were wet with reverse-osmosis water, excess water was removed by rolling on blotting paper, and the swabs were rolled back and forth 4 times, each swab covering a surface area approximately 3 cm by 1 cm. The same surface area was immediately dried by rolling a dry cotton swab over it 4 times. Samples were left to air dry 4 days, then colour measurements were carried out at 37 weeks after the end of the vapour exposure periods, 35 weeks for the Me-2d samples. Photo-documentation was performed 2 days after cleaning.

Following the vapour exposures of Experiment 2, swab cleaning was carried out on AT samples of Bark C that developed a visually noticeable whitish bloom. Cotton swabs

were wet with reverse-osmosis water, excess water was removed by rolling on blotting paper, and the swabs were rolled back and forth a few times until cleaning was achieved. The same surface area was immediately dried by rolling with a dry cotton swab.

RESULTS AND DISCUSSION

Solvent Extractions and Py-GC-MS Analysis

Solvent extractions were carried out on Barks A, B, C, D, E and F to determine which compounds could be solubilized from the bark using methanol or ethanol solvents and, for comparative purposes, water and acetone. Once dry, the extracts were not homogeneous in colour, having whitish and orange residues. As seen in **Figure 2**, both methanol and ethanol solvent extracts had a similar appearance, with significant orange and whitish residues when dried. The dried acetone extract was similar to the methanol and ethanol extracts but with less orange residue, whereas the water extract was less abundant and mainly orange.

Comparison of methanol, ethanol and water homogenized samples of dried extracts

As shown in the total ion chromatograms of **Figure 3** and listed in **Table II**, many of the same compounds were identified in the different extracts, regardless of the solvent or bark specimen. In all cases, the main components were derivatized lupane-type triterpenes (mainly various methyl ethers and methyl esters of betulin, lupeol, betulinic acid, and ursolic acid; 17–23), a result which is consistent with a previously published study.[23] For each bark sample, the methanol and ethanol extracts were similar in composition. The A-water extract, however, was significantly different in its relative abundance of triterpenes (17–21). The A-water extract also contained a relatively high abundance of derivatized fatty acids; the most abundant of which were monounsaturated, including methyl esters of heptadecanoic acid (13), octadecanoic acid (14) and nonadecenoic acid (15). The most abundant saturated fatty acid was the methyl ester of palmitic acid (12). In contrast, only trace to minor relative abundances of palmitic acid and oleic acid were present in the methanol and ethanol extracts.

Other trace to minor components in the methanol, ethanol and water extracts include derivatized phenolic compounds. The most abundant are labelled on the chromatograms in **Figure 3**, and include 3,4-dimethoxystyrene (3), 3,4-dimethoxybenzaldehyde (6) and the methyl esters of 3,4-dimethoxy benzoic acid (8), 4-methoxy cinnamic acid (9), gallic aid (10) and 3,4-dimethoxy cinnamic acid (11). These compounds originate from lignin and hydrolysable tannins in the bark samples. They are widespread in nature and found in low concentrations in plants.[24-26] Additional components identified in the water extract include methyl 3-methoxy butyrate (1), which is the derivatization product of 3-hydroxy butyric acid, a component of plant and animal cells;[27] as well as permethyl-α-d-glucopyranosyl-(1-1)-α-d-glucopyranoside (16), a derivatization product of a glucose-glucose disaccharide.

Traces of several sesquiterpene compounds were detected in the C-ethanol extract and the E-methanol extract. The most prominent sesquiterpenes present in the extracts were identified as α-bergamotene (4) and β-farnesene (5). This is consistent with known sesquiterpene compounds in the bark of *Betula papyrifera* Marsh.[28]

Suberin is a natural polymer that requires alkaline hydrolysis or biodegradation conditions to break apart and release individual polymer units (ω-hydroxy fatty acids, long-chain α,ω-dicarboxylic acids, and mid-chain epoxy or dihydroxy derivatives).[8] The absence of these compounds in the methanol and ethanol extracts likely indicates that hydrolysis has not occurred and that the suberin remains intact in the bark.

Separated whitish and orange fractions of the E-methanol extract

Many of the compounds already described in the extracts of the homogenized samples were present in both the whitish and orange residues from the methanol extract of Bark E.

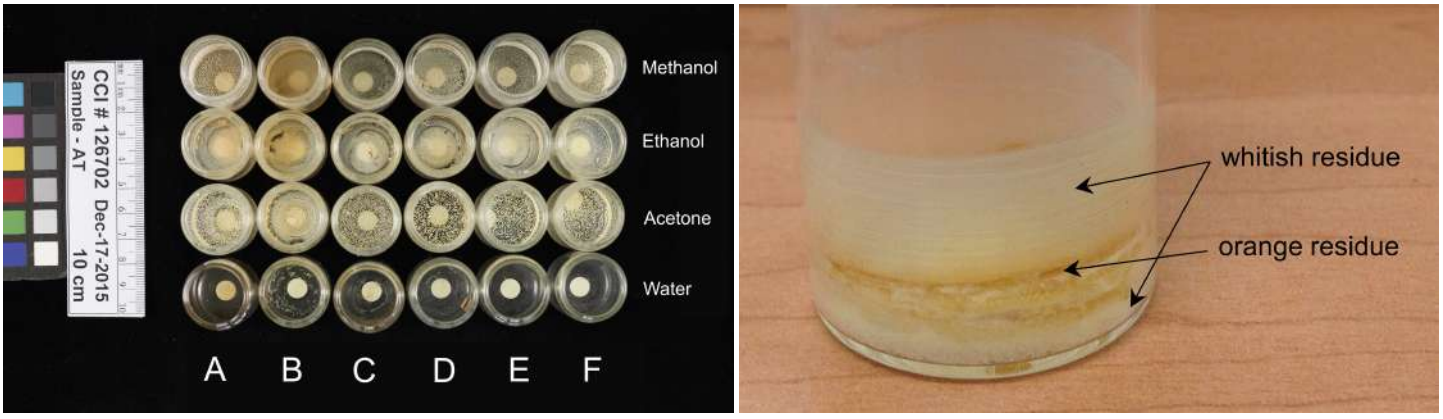

Figure 2. Left: Top view of vials containing dried extracts from bark samples A to F (white dots are stickers placed under the vials). Right: Extract colours, B-ethanol.

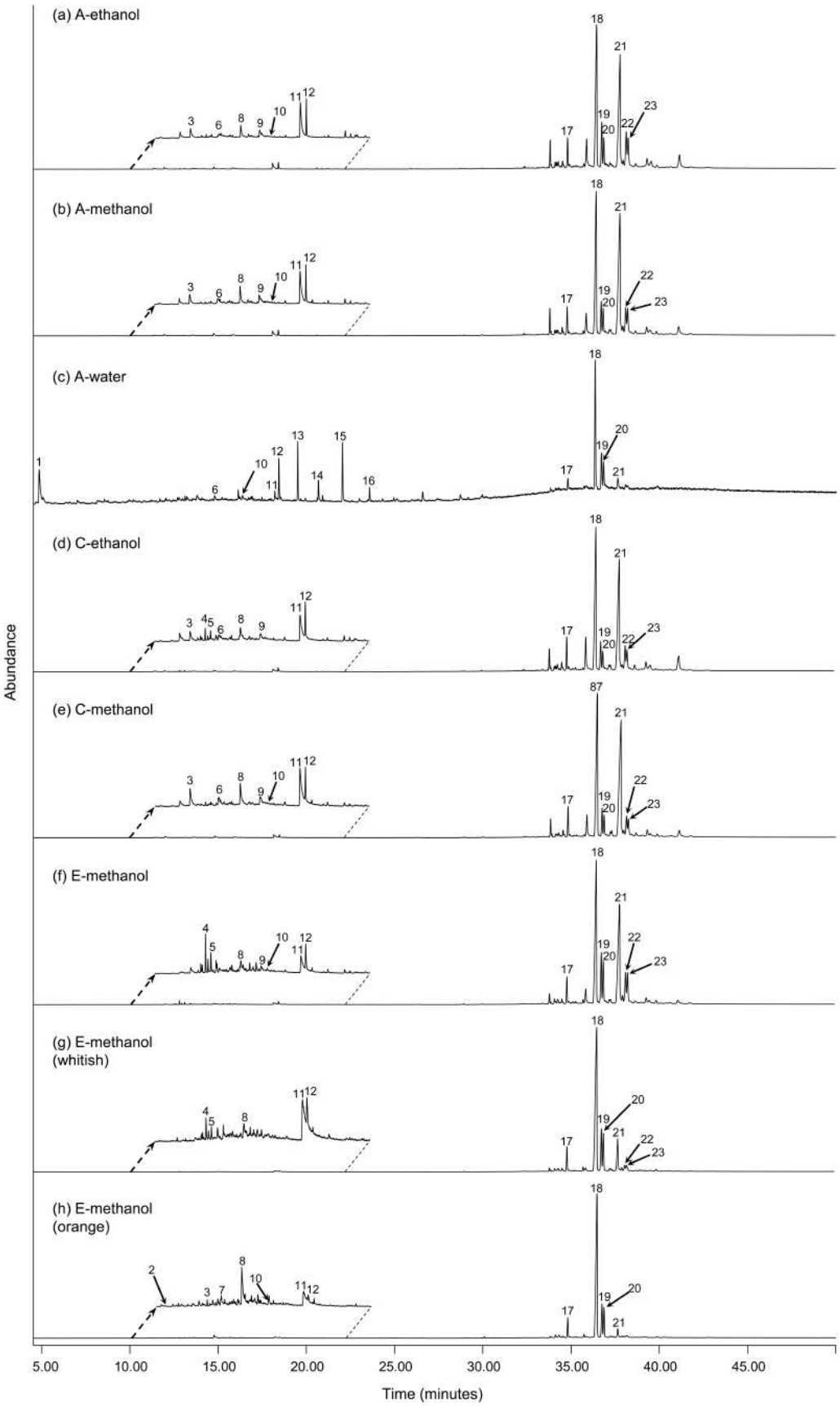

Figure 3. Total ion chromatograms obtained from the Py-GC-MS analysis of methanol extracts (Barks A, C and E), ethanol extracts (Barks A and C), and a water extract from Bark A. Chromatograms for the methanol and ethanol extracts have been expanded from 10–22 minutes to show trace peaks. Peak labels correspond to compounds provided in Table II.

Table II. Components identified using Py-GC-MS for methanol extracts (Barks A, C and E), ethanol extracts (Barks A and C), and a water extract from Bark A.

Component [Peak]‡	Sample (bark – solvent)* A - ethanol	A - methanol	A - water	C - ethanol	C - methanol	E - methanol	E - methanol (whitish fraction)	E - methanol (orange fraction)
Triterpenes:								
lupeol [17]	xx	xx	x	xx	x	x	x	x
betulin [18, 21]	xx	xx	xx	xx	xx	xx	xx	xx
ursolic acid [19, 22]	xx	xx	xx	xx	xx	xx	xx	xx
betulinic acid [20, 23]	xx	xx	xx	xx	xx	xx	xx	xx
Sesquiterpenes:								
α-bergamotene [4]	--	--	--	tr	--	tr	tr	--
β-farnesene [5]	--	--	--	tr	--	tr	tr	--
Phenolic compounds:								
3,4-dimethoxystyrene [3]	tr	tr	--	tr	tr	--	--	tr
3,4-dimethoxy benzaldehyde [6]	tr	tr	x	tr	tr	--	--	tr
3,4-dimethoxy benzoic acid, methyl ester [8]	tr	x	--	tr	tr	x	tr	x
4-methoxy cinnamic acid, methyl ester [9]	tr	tr	--	tr	tr	tr	--	--
3,4-dimethoxy cinnamic acid, methyl ester [11]	x	x	x	x	x	x	x	x
Hydrolysable tannins:								
gallic acid, methyl ester [10]	tr	tr	x	--	--	tr	--	tr
Carboxylic acids (fatty acids):								
hexadecanoic acid, methyl ester [12]	x	x	xx	x	tr	x	tr	x
heptadecenoic acid, methyl ester [13]	--	--	xx	--	--	--	--	--
octadecenoic acid, methyl ester [14]	tr	tr	x	tr	tr	tr	--	--
nonadecenoic acid, methyl ester [15]	--	--	xx	--	--	--	--	--
Carbohydrates:								
permethyl-α-d-glucopyranosyl-(1-1)-α-d-glucopyranoside (glucose-glucose) [16]	--	--	x	--	--	--	--	--
permethyl 3-deoxy-pentonic acid, methyl ester (arabinose) [2]	--	--	--	--	--	--	--	tr
permethyl 3-deoxy-hexonic acid, methyl ester (galactose)[7]	--	--	--	--	--	--	--	tr
Other:								
3-methoxy butyrate, methyl ester [1]	--	--	xx	--	--	--	--	--

*Samples A (ethanol, methanol, water) and C (ethanol, methanol) were each homogenized prior to analysis. For Sample E-methanol, the white and orange fraction were sampled separately and analyzed, then the remaining extract was homogenized and analyzed. Relative abundances (major, minor, or trace) are calculated based on peak area. Compounds with major abundances (xx) have integrated peak areas that are 10–100% relative to the largest peak in the chromatogram. For compounds with minor abundances (x), the range is 1–10%, and trace peaks (tr) have areas that are less than 1% of the largest peak.

‡Peak labels are provided in brackets following the compound names, and these refer to peak assignments provided in Figure 3.

The major components of both were methylated derivatives of the lupane-type triterpenoid resin compounds lupeol, betulin, ursolic acid and betulinic acid (17–21). Trace and minor relative abundances of the methylated phenolic compounds 3,4-dimethoxy benzoic acid (8) and 3,4-dimethoxy cinnamic acid (11) were found in both the whitish fraction and the orange fraction. The orange fraction also contained traces of the phenolic compounds 3,4-dimethoxystyrene (3) and 3,4-dimethoxybenzaldehyde (6). Traces of the sesquiterpenes α-bergamotene (4) and β-farnesene (5) were detected in the whitish fraction, but were not present in the orange fraction. Trace abundances of hydrolysable tannins (gallic acid, 10) and the carbohydrates galactose and arabinose were identified in the orange residue from the presence of permethyl 3-deoxy-pentonic acid, methyl ester (2) and permethyl 3-deoxy-hexonic acid, methyl ester (7),[22] respectively; but these components were not detected in the whitish residue. Galactose and arabinose may originate from the pyrolytic cleavage of arabinogalactans, primary structural components of cell walls and present in all parts of higher plants.[29] Because the whitish and orange fractions were not homogenized, it is possible that the microsamples that were analyzed were not completely representative of the two different coloured fractions. With the exception of gallic acid from hydrolysable tannins in the orange extract, no other coloured compounds were identified in either fraction.

In summary, many compounds can be solubilized and extracted from birch bark using methanol and ethanol solvents. The compositions of the methanol or ethanol extracts

from different birch bark specimens were consistent with those previously studied.[23] No significant differences were observed between the compositions of the methanol and ethanol extracts, although the relative abundances of components varied slightly. Main components in the extracts are the lupane-type triterpenes betulin, lupeol, betulinic acid and ursolic acid. Minor and trace components include fatty acids, phenolic compounds, hydrolysable tannins, sesquiterpenes and carbohydrates. No suberin was detected. The water extract, on the other hand, was significantly different: the relative abundance of triterpenes was lower than that found for the ethanol and methanol extracts, and the fatty acids were greater. The water extract also contained a high relative abundance of 3-hydroxy butyric acid. There were also variations in the colours of the extracts using different solvents. The extracts obtained using either methanol or ethanol were orange and white. In comparison, acetone extracts are similar but less orange, and water extracts, predominantly orange. The orange colour in some extracted material may be in part associated with the presence of hydrolysable tannins. These were identified from the presence of gallic acid in the pyrolysed extract.

Because methanol and ethanol solvents extract the same compounds in birch bark, vapour exposure to these solvents may soften the bark structure and mobilize or react with the same compounds in a similar manner. In particular, both methanol and ethanol extractions mobilize similar amounts of the same triterpene compounds including white betulin compounds, which is consistent with observations that bark exposure to either solvent can sometimes lead to the appearance of a whitish bloom.[2,16] Because water gave different extraction results, water vapour or steam is expected to behave differently, which is consistent with observations that water exposures do not result in the appearance of a whitish bloom.[2,16]

Experiment 1: Barks B, C and E Exposed to Methanol and Ethanol Vapour for Two Exposure Periods

Visual assessment of barks before and after vapour exposures and airing out for 35 weeks

Barks B, C and E before treatment (BT) and after vapour exposure with 35 weeks of airing out (AT-35w Before Cleaning) are shown in **Figures 4**, **5** and **6**, with visual observations summarized in **Table III**. (Barks after cleaning shown in these figures will be discussed below under *Assessment of vapour-exposed bark before and after swab cleaning with water*.) Visually, colour differences between the AT samples of the same bark exposed to different solvents or for different exposure periods were not generally discernible;

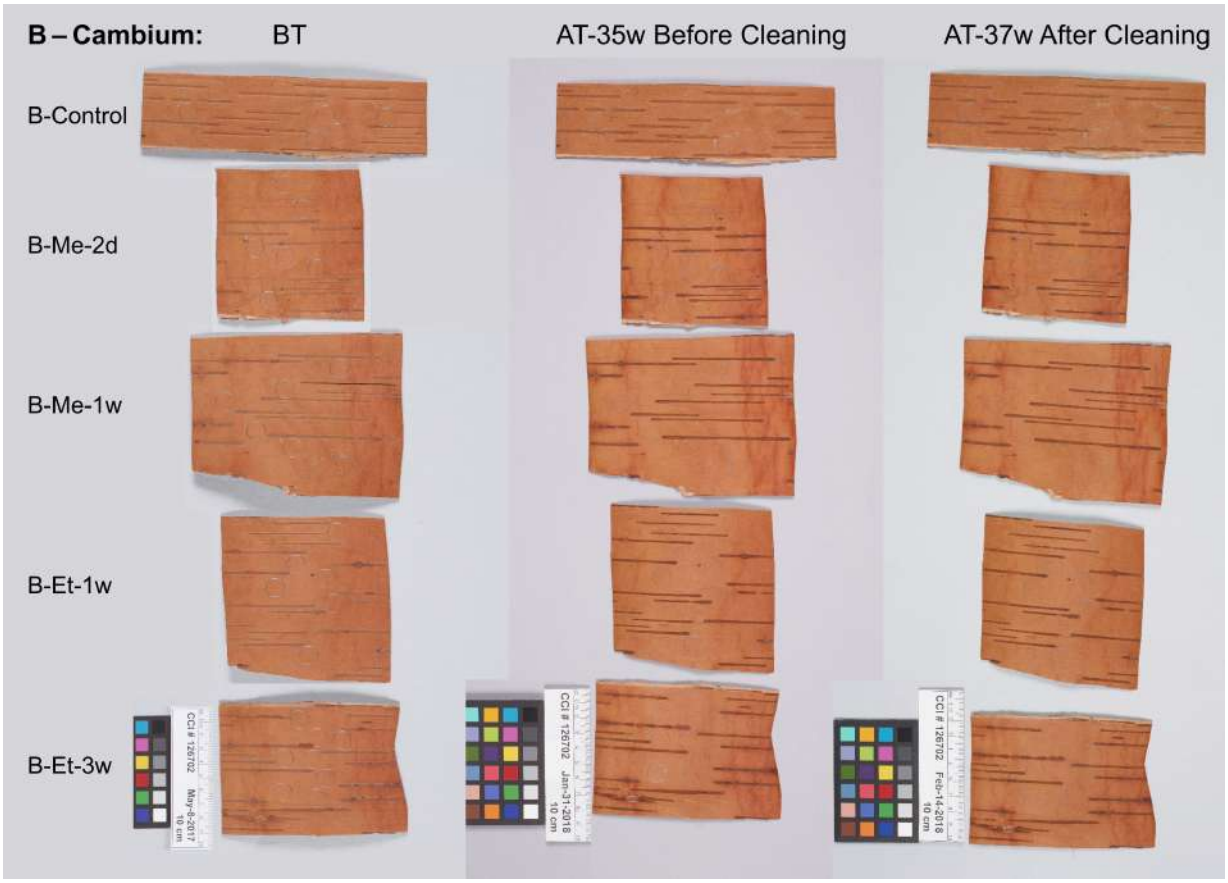

Figure 4. Visual results of the cambium side of Bark B: before treatment (BT), after solvent vapour exposure and 35 weeks of airing out (AT-35w Before Cleaning), and two weeks later after swab cleaning with water (AT-37w After Cleaning).

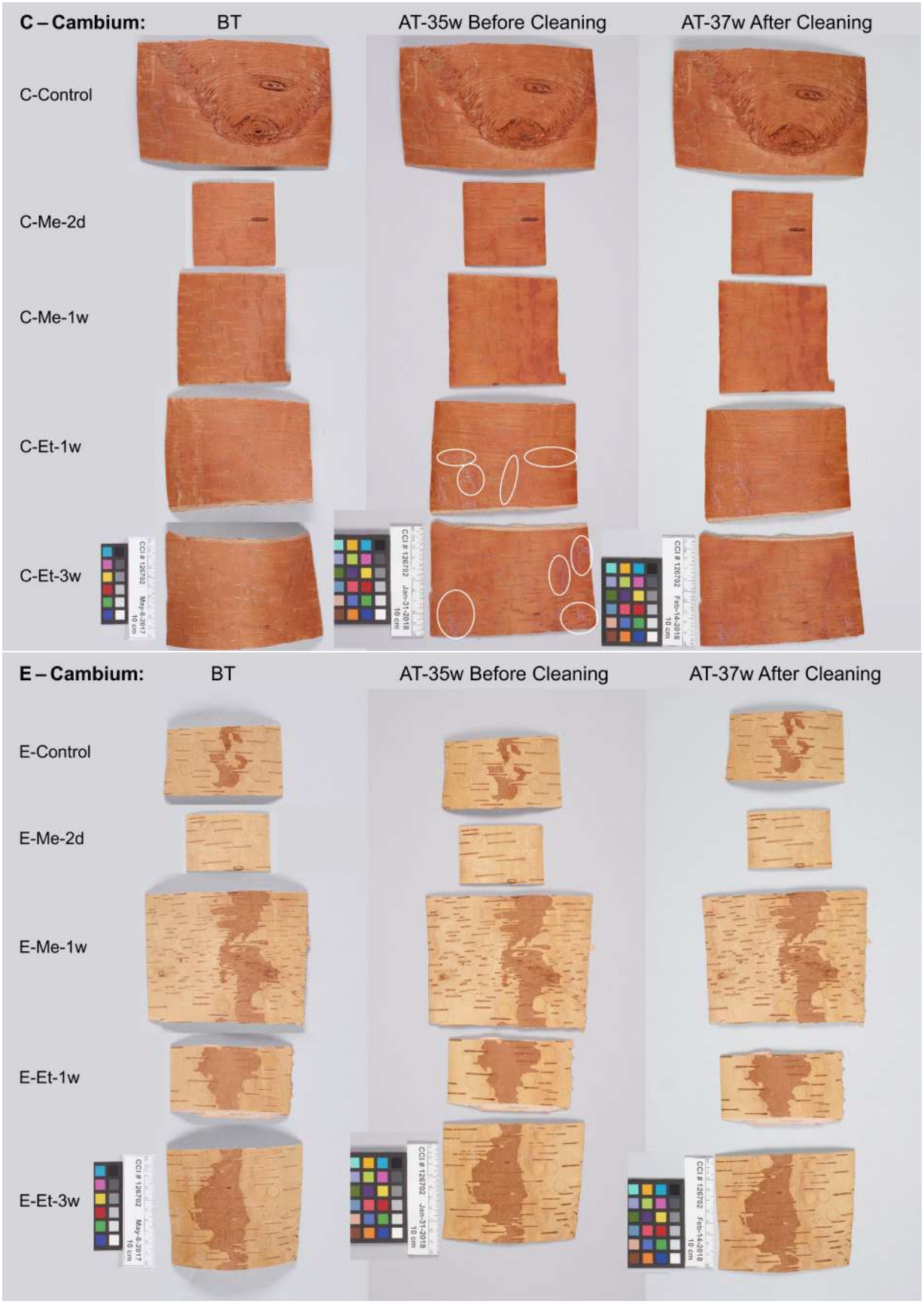

Figure 5. Visual results of the cambium side of Barks C and E: before treatment (BT), after solvent vapour exposure and 35 weeks of airing out (AT-35w Before Cleaning), and two weeks later after swab cleaning with water (AT-37w After Cleaning). The circles on the two ethanol-exposed Bark C samples highlight areas where white deposits occurred due to vapour exposure.

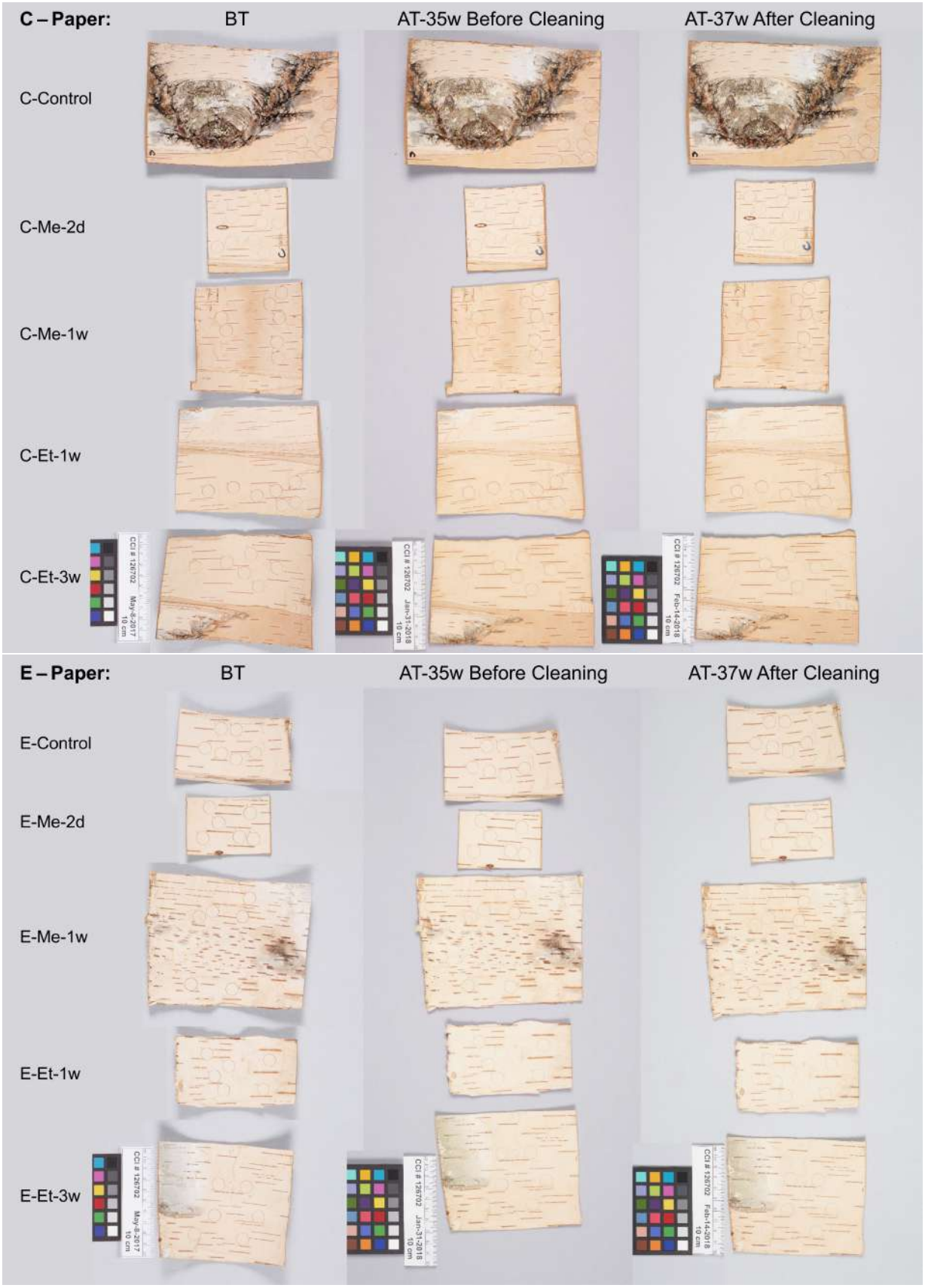

Figure 6. Visual results of the paper side of Barks C and E: before treatment (BT), after solvent vapour exposure and 35 weeks of airing out (AT-35w Before Cleaning), and two weeks later after swab cleaning with water (AT-37w After Cleaning).

Table III. Summary of visual and colorimetric results after vapour exposure followed by airing out 35 weeks and before cleaning. White = below perceptibility threshold of ΔE=1.5. Light grey = at perceptibility threshold ±0.2 unit. Darker grey = above perceptibility threshold.

			Total Colour Change (ΔE) and Description*				
	Bark	Visual Results	Methanol-2.3 days	Methanol-1 week	Ethanol-1 week	Ethanol-3 weeks	Control
Cambium side	B (brown)	Lighter and possibly redder; darker lenticels	1.2	1.6 lighter, yellower	2.3 lighter	3.6 lighter, less yellow	0.3
	C (reddish orange)	Slightly redder and/or darker; some white deposits on the ethanol-exposed samples	2.7 darker, redder	3.1 darker, redder	1.6 redder	2.8 less yellow	0.6
	E (beige)	No noticeable visual change	0.8	1.7 darker	1.1	0.8	0.3
Paper side	C	Slightly lighter and/or yellower	2.3 lighter, yellower	2.2 lighter, yellower	2.3 lighter, yellower	2.5 lighter, yellower	0.2
	E	Slightly lighter and/or yellower	1.4	2.3 yellower	1.7 lighter, yellower	1.9 yellower	0.3

Description gives which components among L, a* or b* most contribute to the measured total colour change (ΔE): lighter = increase in L*, darker = decrease in L*, yellower = increase in b*, less yellow = decrease in b*, redder = increase in a*.

however, some differences between the BT and AT samples were discernible. The perceived changes were generally deemed not very conspicuous ("slight"…) and only imprecisely described ("possibly…", "and/or"). Distinguishing nuances in colour changes can be difficult for the eye, especially when lighting and background are not strictly consistent.[30] Unlike the colorimeter, visual assessments tend to scan whole samples and not specific spots, which may visually blur out some colour changes since the overall colour of each bark sample is not uniform, but covers a broad range. The visual assessments were valuable in discerning conspicuous changes occurring in areas outside of the colorimetric test spot, such as the presence of some white specks that were observed on Bark C exposed to ethanol vapour (see circled areas in **Figure 5**, shown in detail in **Figure 7**) whereas none were visible on the methanol samples. Also of note is the darkening of Bark B's lenticels after exposure to both solvent vapours (**Figure 4**, see also **Figure 14**) which gave an impression of increased contrast between light and dark areas of the bark.

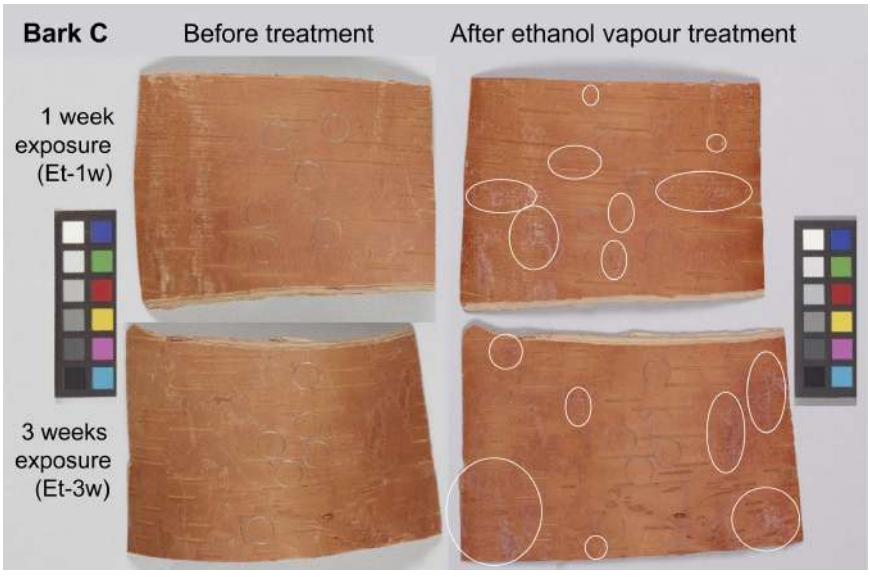

Figure 7. Detail of the white specks of bloom that appeared on Bark C after ethanol vapour exposures (see circles).

Colorimetric assessment of barks before and after vapour exposures and airing out for 35 weeks

Table III presents a summary of the colorimetric results for the three barks at the end of the airing out period and before cleaning. In this study, a ΔE of 1.5[30] (indicated through grey shading in **Table III**) is used as the threshold for a visually perceptible colour change.[31] In general, the extent and type of colour change (increase in L* (lighter), increase in b* (yellower), etc.) indicated by colorimetry are consistent with the visual descriptions. The colorimetric results, however, reveal differences between samples of the same bark exposed to different solvents and exposures, and give more detailed information on the extent and type of colour change (lighter/darker, redder/less red, yellower/less yellow). The data show that

the shorter exposures to methanol (2.3 days) and to ethanol (1 week) caused less colour change in general than longer exposures, although visually this was not apparent. Colorimetry also showed differences between the ethanol and the methanol "shorter" exposure, with the methanol 2.3-day exposure giving equal or less pronounced colour changes than the ethanol 1-week exposure for 4 out of 5 surfaces measured.

The colorimetric data confirm that the colour of each bark is not uniform but rather, varies across the sample's surface. **Figure 8** shows this range of colour (range of L*, a* and b* values) for the cambium side of the three barks B, C and E. The BT data shows the initial range of colour for all spots on all samples, while the AT colour range, after either methanol or ethanol vapour exposure, combines the two exposure periods. As seen in **Figure 8**, the AT colour readings often remained within the BT colour range. This may explain why colour changes were often hard to discern visually: if the AT colour readings for a particular bark fall within its BT range, any differences may be masked by the bark's heterogeneous colour and so may not be very noticeable or conspicuous to the eye.

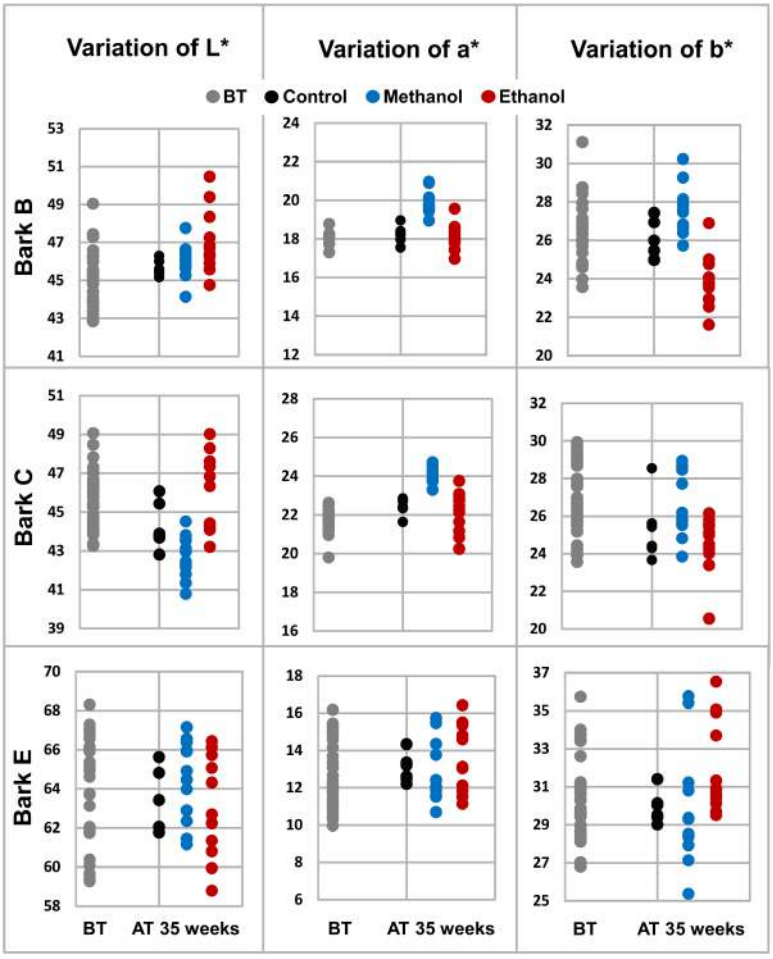

Figure 8. Variation of L*, a* and b* values of the cambium side for Barks B, C and E before and after solvent vapour exposures followed by 35 weeks (245 days) of airing out.

Details of the colorimetric measurements for Barks B, C and E taken before treatment (BT), immediately after vapour exposure (shown as T=0) and over the course of airing out to up to 35 weeks (AT-35w) are shown in **Figure 9** for the cambium side and in **Figure 10** for the paper side. Several points can be drawn from the graphs, as discussed below.

Cambium side (see **Figure 9**)

Controls: The average colour change for the controls at the end of the experiment (35 weeks) is well within ΔE of 1.5 units (just perceptible colour change), and all are under 0.5 except those for the Bark C control (below 1.0). This is similar to previous results from other samples of these same 3 barks after 1 year of dark ageing in CCI's environmentally controlled laboratory: for 2 control samples, ΔE measured 0.1 unit on each for Bark B; 0.2 and 0.3 units for Bark E; and 0.3 and 0.4 units for Bark C.[6]

After methanol vapour exposures plus 35 weeks of airing out: The cambium side of all barks changed colour after methanol vapour exposure and airing out, but to significantly different degrees. The reddish orange Bark C had the highest colour change, in the range of ΔE~3, which is above the perceptibility threshold (of 1.5) for both exposure periods; most of this colour change was due to a reddening (increase in a*) and darkening (decrease in L*). The colour change for Barks B and E was below the perceptibility threshold in the case of the short Me-2d exposure, and just barely above the perceptibility threshold (at 1.6) in the case of the long Me-1w exposure (although at Time=0 before airing out it was initially significantly higher). The colour change for both of the two darker barks, B and C, was characterized by reddening (increase in a*), most pronounced in the reddish orange Bark C. Their changes in L* and b*, however, were different: Bark B lightened (increase in L*) and yellowed (increase in b*) while Bark C darkened (decrease in L*) and lost yellow (decrease in b*).

After ethanol vapour exposures plus 35 weeks of airing out: The cambium side of all barks changed colour after ethanol vapour exposure and airing out, but to significantly different degrees. The most significant visually perceptible colour change was with the brown Bark B at the two exposure periods (ΔE=2.3 for Et-1w and 3.6 for Et-3w), as well as with the reddish orange Bark C at the long Et-3w exposure (ΔE=2.8). Colour change for Bark B was due mainly to a lightening (increase in L*); for Bark C, colour change for the Et-3w exposure was mainly due to a loss in yellow (decrease in b*) and for Et-1w, mainly to a reddening (increase in a*). For the pale beige Bark E, ΔE was below the perceptibility threshold for both ethanol exposures.

Comparison of methanol to ethanol vapour exposures after airing out for 35 weeks: Both solvents generally affected the darker Barks B and C more than the paler beige Bark E. Ethanol affected Bark B more than methanol vapour: the ethanol

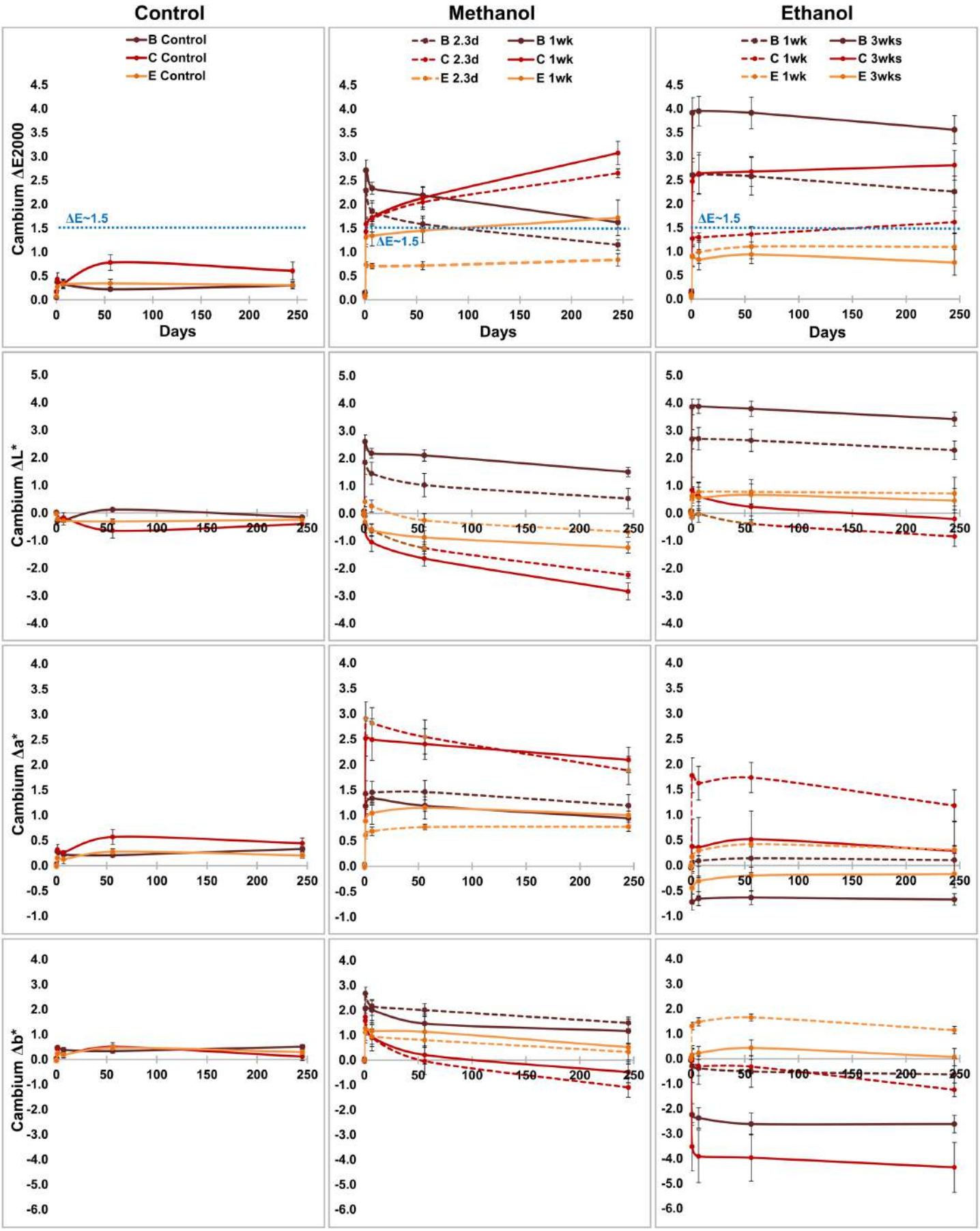

Figure 9. Colorimetric results for cambium side of Barks B, C and E after two different methanol or ethanol solvent vapour exposures from time=0 to up to 35 weeks (245 days) of airing out.

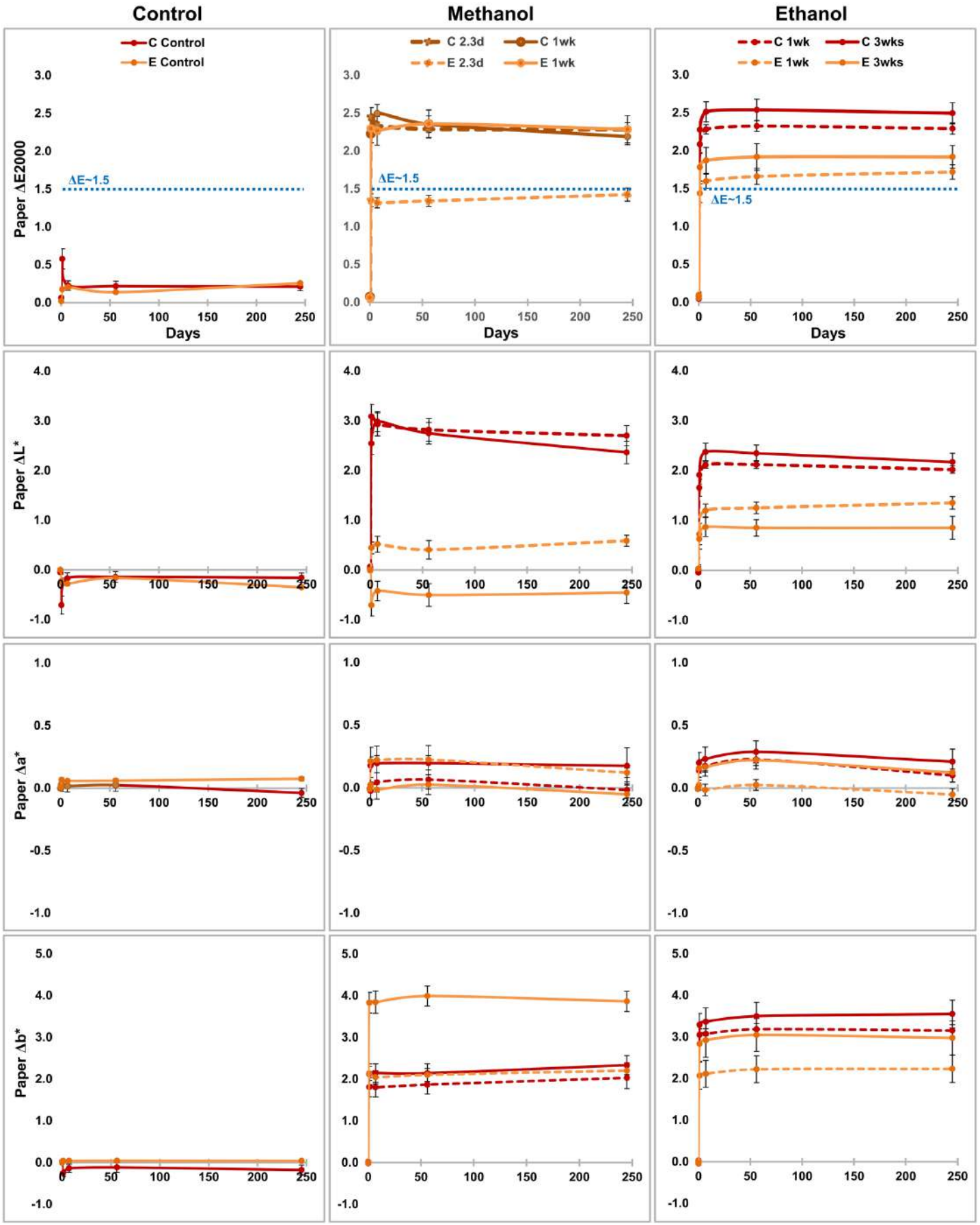

Figure 10. Colorimetric results for the paper side of Barks C and E after two different methanol or ethanol solvent vapour exposures from time=0 to up to 35 weeks (245 days) of airing out.

exposures caused almost twice as much colour change as methanol. On the other hand, both exposures to methanol vapour affected Bark C strongly, while only the longer ethanol vapour exposure (Et-3w) had a similarly strong effect; the shorter Et-1w exposure had a ΔE about half as large and just barely above the perceptibility threshold. For the beige Bark E, both methanol and ethanol vapours caused only minor colour change, in the range of the perceptibility threshold (just below or just above). For all barks, the changes in L* are similar for both methanol and ethanol: for Bark B it increased, for Bark C it decreased, and for Bark E it did not vary much. Changes in the hues a* and b* were different depending on the solvent, with the darker Barks B and C exposed to methanol vapour typically becoming slightly redder (higher a*) than those exposed to ethanol vapour.

Effect of longer solvent vapour exposures: For both solvents, longer exposures produced larger ΔE and correspondingly larger changes in L*, a* and b* values, except for ethanol and Bark E, where the changes were comparable. For the darker Barks B and C, the gap between the colour change of the short and long ethanol exposures was wider than the gap between the two methanol exposures (though also there was a larger time gap between the short and long exposures for ethanol versus the time gap of the two methanol exposures). This wider gap in the two ethanol exposures' colour change correlated well with their wider gap in weight gain as compared to the two methanol exposures (as seen in **Table IV**).

Effect of airing out: This effect was significant only in the case of the two darker Barks B and C when exposed to methanol vapour, as their colour change after airing out 35 weeks was significantly different (by over 1 unit of ΔE) from that measured immediately after the end of the vapour exposure (Time=0). It is interesting to note that for the brown Bark B, the colour change significantly *decreased* during airing out with changes occurring most rapidly during the first week, and ended up at or below the perceptibility threshold (ΔE went from 2.3 to 1.2 for Me-2d and from 2.7 to 1.6 for Me-1w); while for the reddish Bark C, the colour change was first at the perceptibility threshold after 1 day of airing out, but then progressively *increased* at a fairly steady rate (for Me-2d it went from 1.6 to 2.7, for Me-1w, 1.4 to 3.1). In comparison, colour remained essentially constant (within experimental error) during airing out for all barks exposed to ethanol vapour, as well as for beige Bark E exposed to methanol vapour.

Paper side (see **Figure 10**)

Controls: Colour change for controls was within ΔE of 0.5 for all barks and thus below the perceptibility threshold.

Comparison of methanol to ethanol vapour exposures after airing out for 35 weeks: The paper side of Barks C and E exhibited colour changes that consisted of lightening and yellowing when exposed to both methanol and ethanol. For Bark C, the results were similar for both solvents at both exposures and most pronounced (ΔE~2.4), with ethanol vapour causing more yellowing but less lightening than methanol vapour. There was no noticeable reddening (no increase in a*). For Bark E the colour change was perceptible with the longer methanol exposure, Me-1w (ΔE=2.4), but not with the shorter exposure, Me-2d (ΔE=1.4), while it was just above the perceptibility threshold with both ethanol exposures (ΔE=1.9 with Et-3w and 1.7 with Et-1w). Again the change was usually due mainly to lightening and yellowing, except for Me-1w which showed a strong yellowing combined with a small amount of darkening and reddening.

Effect of longer exposures: Longer exposures did not strongly affect the colour change on the paper side: only the longer methanol vapour exposure (Me-1w) for the beige Bark E produced a statistically significant greater colour change than the corresponding shorter exposure.

Effect of airing out: For both Barks C and E, a large colour change was measured at the start of the drying period (Time=0) for both solvents, a colour change that remained relatively stable during airing out (variations are within experimental error).

Weight gain

The change in weight over time for each bark of Experiment 1 is shown in **Figure 11**. Measurements up to T=0 correspond to the weight increase due to vapour absorption during the exposure treatment, while those following T=0 correspond to vapour desorption during drying and airing out. As can be seen for the three barks exposed to ethanol for 3 weeks (Et-3w), where measurements were taken every week over the course of the three week exposure (before T=0), vapour absorption was not linear in time: the increase in weight was steeper at the beginning of the exposure and gradually tapered off as the bark had less capacity to absorb more vapour. When vapour exposures stopped and the barks started drying (T=0 onwards), their weight decreased rapidly, indicating fast vapour desorption. After one week of drying (between blotters and boards) the weights of ethanol-exposed bark samples remained approximately 5% higher than their respective BT weights, whereas the weights were only 3% higher for the methanol-exposed barks. As the barks were subsequently left to air dry, the bark weights continued to progressively decrease but at a slower rate. It took several weeks for complete evaporation: with methanol vapour, the weight gain was negligible (±1%) after 8 weeks, while with ethanol, low levels of solvent vapour lingered longer. This is consistent with methanol being more volatile than ethanol and is similar to previous results.[5]

Table IV gives the peak % relative weight (ATweight/BTweight in %) for each bark sample at the end of each vapour exposure period (i.e., at T=0), which was the time of peak absorption. With ethanol vapour, the longer exposure period (Et-3w) resulted in a higher weight gain, which is expected since the bark gradually absorbs more vapour over time. With methanol vapour, however, neither Bark B nor Bark E increased substantially in weight after 2.3 days of exposure (differences were within experimental error). This indicates that methanol absorption had already begun to plateau after 2 days. As seen in **Figure 9**, the colour change for Barks B and E exposed to methanol for 1 week (Me-1w) was nevertheless *greater* than that of the sample exposed

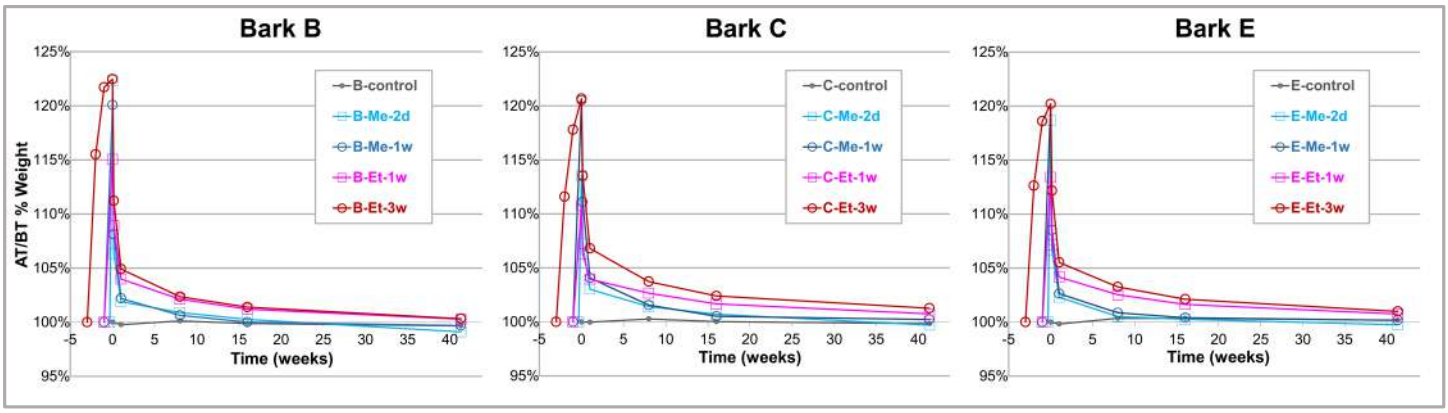

Figure 11. Change in bark weights due to vapour exposure and airing out for Barks B, C and E (left to right). AT weight/BT weight expressed in % where 100%=BT weight. Before T=0, the barks were exposed to the solvent vapours for a set period (Me-2d=for 2.3 days; Me-1w=for 1 week; Et-1w=for 1 week; Et-3w=for 3 weeks). Time=0 indicates the end of the vapour exposure period and start of drying; it is also when the barks show maximum weight gain due to their absorption of vapours. After T=0, the barks were progressively airing out.

for 2.3 days (Me-2d) even though the amount of methanol vapour absorbed (the weight gain) was similar. This suggests that exposure time may play a role in colour change that is independent of the quantity of vapour absorbed within the bark (weight gain): a longer vapour exposure period could provide more time for chromophoric compounds to develop or migrate to the surface.

Of samples exposed to solvent vapour for the same lapse of time (1 week), all ethanol-exposed barks had a significantly lower weight gain than those exposed to methanol vapour, which concurs with previous findings.[5] Exposure to ethanol vapour for three weeks (Et-3w) achieved a weight gain similar to that achieved by a 1-week exposure to methanol vapour (Me-1w). As for the shorter exposure times, the 1-week ethanol exposure (Et-1w) produced less weight gain than just 2.3 days of exposure to methanol (Me-2d). This difference in the effect of methanol and ethanol vapours on birch bark is even greater if the difference in molecular weight (46.07 g/mol for ethanol versus 32.04 g/mol for methanol) is considered: even with identical weight gains, there would be approximately 1.4 times more molecules of methanol absorbed than ethanol.[5] The faster methanol absorption rate is probably linked to its higher vapour pressure (12.98 kPa for methanol versus 5.95 kPa for ethanol at 20°C[32]): there are more methanol molecules in equilibrium in an enclosed space (vapour bag) under equivalent conditions. Methanol is also a smaller molecule than ethanol, which may allow faster absorption into birch bark.

Comparing these results with those of previous research on weight gain and reshaping[5] leads to the following observations:

Methanol vapour exposures: The previous study found that barks with a weight gain of around 14% could probably be successfully reshaped. Therefore the 20% weight gain achieved in the current experiments after a 1-week exposure may be beyond what is necessary for reshaping and, as seen in **Figures 9** and **10**, often results in significant colour changes. The shorter methanol exposure period of 2.3 days also gave, for Barks B and E, higher weight gains than may be needed for reshaping (22% and 19% respectively); thus it is conceivable that an even shorter exposure period could yield both effective reshaping and less colour change. Interestingly, with the short 2.3-day methanol vapour exposure, Bark C had the lowest weight gain (only 14%) compared to the other 2 barks yet also exhibited the greatest colour change. This suggests that a bark's original colour (in this case, a reddish tone as opposed to brown or beige colours) has a significant impact on the degree of colour change due to solvent vapour exposure. Since the lower weight gain threshold for successful reshaping with methanol vapour is not known, it remains possible that for this bark too, an exposure shorter than 2.3 days and/or a weight gain lower than 14% could achieve sufficient pliability for reshaping with a less pronounced colour change.

Table IV. Weight of barks relative to their BT weight (AT weight/BT weight expressed in %) at end of solvent vapour exposures (at T=0), just before the start of airing out. 100%=same as BT weight. The % that is beyond 100%= the weight *gain*.

Solvent:	Methanol vapour		Ethanol vapour	
Exposure time:	2.3 days (Me-2d)	1 week (Me-1w)	1 week (Et-1w)	3 weeks (Et-3w)
Bark B	122%±1%	120%±1%	115%±1%	122%±1%
Bark C	114%±1%	121%±1%	111%±1%	121%±1%
Bark E	119%±3%	120%±1%	113%±2%	120%±1%

Note: The ± values are a calculated error for one sample not a reflection of variation among repeated samples.

Ethanol vapour exposures: The previous study found that, for the barks tested, a 17% weight gain resulted in successful reshaping but a 9% weight gain did not.[5] In this current study, the longer, 3-week ethanol exposure resulted in weight gain of a 20% or more (which might therefore be excessive for reshaping treatment needs) and produced relatively higher colour changes than with the shorter exposure for the two darker Barks B and C. The shorter, 1-week ethanol vapour exposure resulted in moderate weight gains (11%, 13% and 15%), possibly conducive to reshaping, yet colour changes remained at or above the perceptibility threshold on the cambium side for the two darker barks and on the paper side for the lighter bark, Bark E. Further experiments are needed to see if a shorter exposure and/or lower weight gain could be found that would minimize colour changes yet still be conducive to successful reshaping.

Assessment of vapour-exposed bark before and after swab cleaning with water

Cleaning with moistened swabs was carried out on the vapour-exposed barks of Experiment 1 followed by colorimetric measurements in order to determine whether it would alter the colour change after vapour exposure; for example, by removing deposits or solubilized coloured compounds from the surface, or as a result of exposure to water.

Visual assessment of cleaning

Barks before and after swab cleaning are shown in **Figures 4**, **5** and **6** (see centre "AT-35w Before Cleaning" and right "AT-37w After Cleaning" of each image). For the three barks tested, each side showed a similar appearance before and after cleaning. Cleaning removed some but not all of the sparse white deposits that appeared on Bark C after ethanol exposure (**Figure 12**). Note that only a light cleaning (4 rolls) was carried out; it is probable that more deposits could be removed with further cleaning. Note also on **Figure 12** that virtually no white deposits formed on the spots where colorimetric measurements were taken (see graphite circles).

Colorimetric assessment of cleaning

The colorimetric results of the vapour-exposed barks of Experiment 1 before versus after swab cleaning are shown in **Figure 13**. The "before" bars on the graph indicate the existing colour change due to the solvent exposures. The adjoining bars on the graph ("after swabbing," paler in colour) show the effect of subsequent swab cleaning with water. Results show that swab cleaning with water did not have a statistically significant effect on the barks' surface colour (any colour changes are small and within statistical error). This means that the colour changes resulting from vapour exposures are not reversed by light cleaning with a moistened swab, and also that the small amount of water used did not affect the barks' colour. Although cleaning removed some tiny white deposits from the surface of the ethanol-vapour-exposed Bark C, the colour remains essentially the same before and after cleaning because the white deposits were not located on the colour measurement test spots (**Figure 12**). The only statistically significant colour change after swab cleaning was found on the cambium side of Bark B exposed to ethanol vapour (**Figure 14**): ΔE for Et-3w went from 3.6 ± 0.3 to 2.5 ± 0.3, and for Et-1w, from 2.3 ± 0.2 to 1.7 ± 0.2 with the bark's colour components (L*, a*, b*) reverting to values closer to their BT values. In other words, swab cleaning lessened the colour change that had arisen due to the ethanol vapour exposure. This measureable colour change may be due to the removal of a thin, visually imperceptible layer of deposits that is specific to Bark B or more easily removed by swab cleaning from its surface.

Experiment 2: Barks A, C and E after 1-Week Methanol and Ethanol Vapour Exposures

A second series of vapour exposures was carried out on three sets of bark samples: Barks A, C and E. The purpose was to further investigate the occurrence of a white bloom and its cleaning, as traces of a white deposit were found after solvent exposure on one bark (Bark C) in Experiment 1.

Visual assessment

Figure 15 shows the visual results of the barks' cambium side BT and AT for Experiment 2. For Barks A and E, visually no colour change is discernible between BT and AT photographs of samples. Bark C however does appear slightly redder or darker after exposures to both methanol and ethanol vapour. As well, a thin white surface deposit appeared on the two Bark C samples exposed to ethanol vapour (**Figure 16** and

Figure 12. Bark C exposed to ethanol vapour for 1 week (Et-1w) and 3 weeks (Et-3w), before and after swab cleaning.

Figure 13. Colorimetric results for Barks B, C and E after solvent vapour exposures and airing out, before and after swab cleaning.

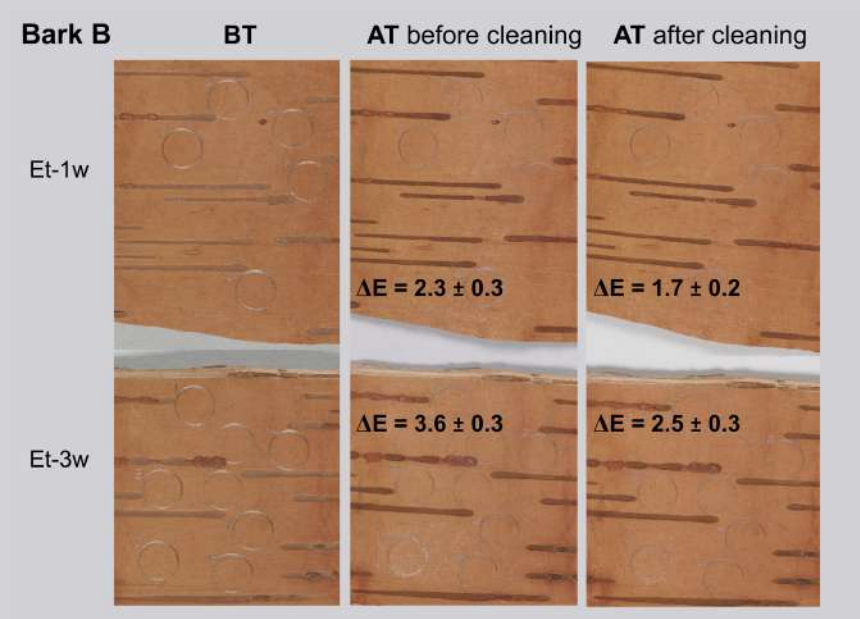

Figure 14. Detail of Bark B exposed to ethanol vapour for 1 week (Et-1w) and 3 weeks (Et-3w). BT=before vapour exposure. AT before cleaning=after vapour exposure. AT after cleaning=after vapour exposure and swab cleaning with water. The colour change ΔE gives the value of the bark colour AT before cleaning and AT after cleaning with respect to the BT bark colour.

Figure 17 left). The two methanol Bark C samples did not show this whitish bloom, nor did Barks A and E samples following either methanol or ethanol vapour exposures.

Py-GC-MS analysis of white surface deposits

Analysis of the white crystals from one sample of Bark C exposed to ethanol vapour (sample C-Et-T2; see **Figure 15**) found that they consist of betulin and lupeol in approximately a 2:1 ratio. These results show that triterpene compounds migrated from the interior of the bark to the outer surface when exposed to ethanol vapour for an extended period. It is interesting to note that the 2:1 ratio of betulin and lupeol found for the surface crystals differs considerably from the ratios of betulin and lupeol found for the liquid ethanol extracts, which were calculated to be 9:1 and 8:1 for Bark A and Bark C, respectively. The reason for decreased betulin to lupeol content in the surface crystals in comparison to the liquid extracts may be the greater molecular weight and polarity of betulin relative to lupeol. These factors may impede the mobility of betulin relative to lupeol when the compounds are exposed to ethanol vapour, but perhaps do not limit the mobility of betulin to the same extent in liquid ethanol. However, these ratios are based on minimal sample analysis, and further research and sampling would be required to determine if they accurately represent the liquid- and gas-phase migration trends for betulin and lupeol.

Swab cleaning with water of the surface deposits

A swab lightly moistened with water was rolled over the surface of Bark C several times to determine how readily the white surface residues could be removed (**Figure 17 centre**). The deposits were easily removed after a few passes (**Figure 17 right**).

CONCLUSIONS

Through a series of related experiments, this study compared the degree to which methanol and ethanol vapour treatments cause colour changes to birch bark. Solvent extracts from birch bark were analyzed to determine their composition. Colour change and weight gain of samples exposed to methanol and ethanol vapours were measured. Finally, the effect of swab cleaning of the surface with water was assessed.

Regardless of bark colour, immersion of samples in either methanol or ethanol resulted in the solubilization of a similar range of white and orange compounds, including betulin and related triterpenes and minor or trace amounts of fatty acids, phenolic compounds, hydrolysable tannins, sesquiterpenes and carbohydrates. Suberin, an elastic polymer associated with birch bark's natural flexibility, was not detected in the extracts. The orange colour in some of the extracts is likely due to traces of hydrolysable tannins. Water extraction showed a lower abundance of triterpenes and a higher abundance of fatty acids, which is consistent with the observation that treatments using water vapour or steam differ in their effectiveness and visual result (no appearance of any white bloom) from those using methanol, ethanol and other organic solvents.

Barks exposed to either methanol or ethanol vapour showed colour changes, sometimes above the perceptibility threshold defined as ΔE=1.5, reaching at most up to 3.6 units. The colour change depended on the original colour of the bark, the solvent used and the exposure period. For the three barks tested, the colour change on the cambium side was generally more pronounced on the barks with darker coloured cambium sides. It should be noted that the bark samples in these tests were of relatively recent origin, which discolour more than older barks after solvent vapour exposure, according to Gilberg.[2]

The degree of colour changes depended on the solvent and the length of vapour exposure, with longer exposures typically producing a larger colour change. When the results for both cambium and paper sides of the 3 barks studied are considered together, the 2.3-day methanol exposure gave the best results, causing a perceptible colour change in only one of the three barks tested (the reddish-toned Bark C). In comparison, the best ethanol result was the 1-week vapour exposure, which produced a perceptible colour change on at least one side of all three barks (though only at the threshold level for Bark E). For the paper side, the colour change usually consisted of both lightening (increase in L*) and yellowing (increase in b*). The changes in L*, a* and b* were more complex for the cambium

Figure 15. Cambium sides of Barks A, C and E before and after treatments. Et-T1, Et-T2, Me-T1 and Me-T2 refer to one-week exposure to methanol or ethanol vapour; T1 and T2 refer to the flattening regime after vapour exposure. T1 = samples were flattened one week under blotters and weights then aired out freely; T2 = same but flattened eight weeks. W1 refers to the weight applied during flattening (here W1 was used for all samples).

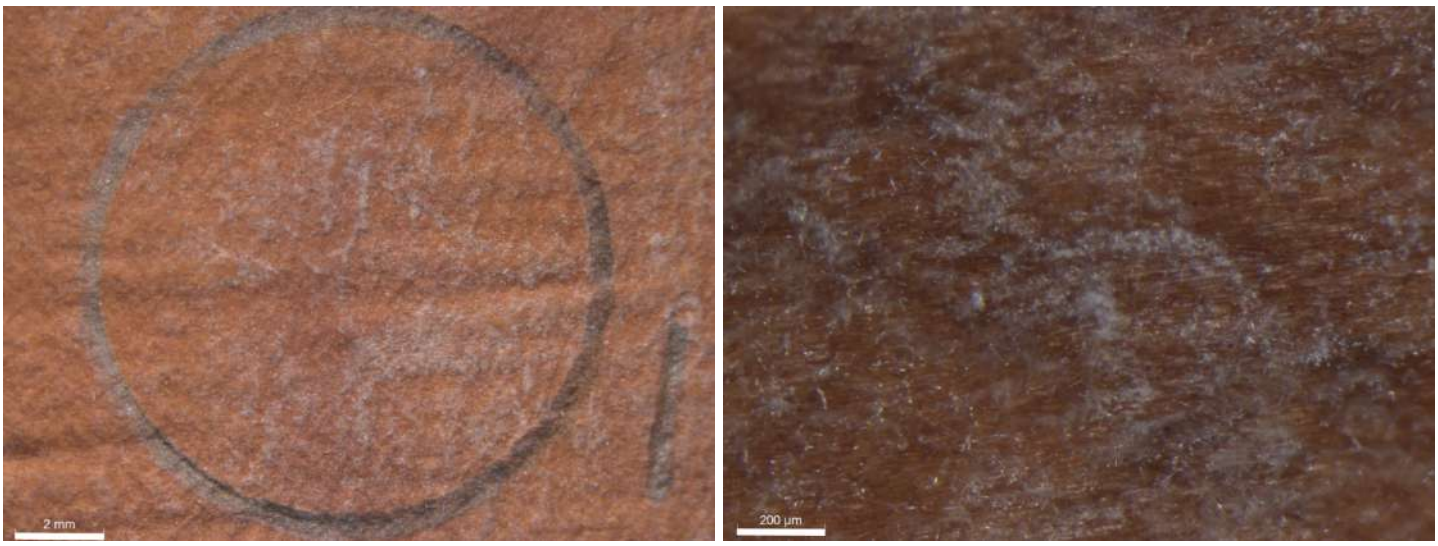

Figure 16. Left: Detail of circle 10 on Bark C sample C-Et-T2 showing whitish deposits. Right: Detail of surface deposits, showing rod-like crystals.

side and depended on the original bark colour and on the solvent used. Visually, the colour changes measured beyond the perceptibility threshold (ΔE of 1.6 to 3.6) were discernible but often not strongly conspicuous to the eye, probably because the colour changes blended in within a bark sample's natural range of colour tones. With ethanol vapour, the colour change measured immediately after vapour exposure remained stable over time for all three barks (the colour did not change further after airing out for 35 weeks). With methanol vapour, however, colour continued to change, increasing over time in the case of the reddish orange bark C but decreasing over time in the case of the brown bark B.

Monitoring the weights of the bark samples during vapour exposure showed that the weight increased as solvent vapour was absorbed during exposure, and decreased as the solvent desorbed during airing out, in both cases in an exponential manner (faster at the beginning and slowing down with time). Methanol vapour absorbed into the barks faster than ethanol vapour: the 2.3-day methanol vapour exposure resulted in a higher weight gain than the 1-week ethanol exposure. Methanol also desorbed faster during airing out, with minimal measurable weight gain after approximately eight weeks of airing out; barks exposed to ethanol vapour took two to five times as long to desorb the solvent and return to their original before-treatment weight. There was considerable variability among the three barks' peak weight gain for a given solvent and vapour exposure period. This was probably due to natural variations in the barks' thickness, size, density, and also knots and number of lenticels (which act as pores).

Regular measurement of bark weight gain during a vapour exposure treatment is recommended as this indicates how well the bark is absorbing the solvent vapour (and thus becoming more pliable for reshaping) as well as the increasing risk of colour change. The duration of vapour exposure also appears

Figure 17. Bark C after one week ethanol vapour exposure (sample C-Et-T2). Left: Some whitish deposits can be seen on the sample (along the bottom and top of the sample and on most of the lower right quadrant). Centre: Swab cleaning was carried out in an area of the lower right quadrant using reverse osmosis water. Right: After swab cleaning, the whitish deposits in that area were effectively removed (see hatched circle).

significant: a higher colour change was measured in two of the three barks (B and E) exposed to methanol vapour for the longer period even though the weight gain was similar to that of the shorter exposure. Thus even if weight gain is stable, longer exposures may provide more time for colour change mechanisms, whether compound migration in the bark or other processes, to occur and should be avoided if possible.

Two other visual effects were found which show the variability between different barks. First, the lenticels on a brown bark (Bark B) were found to have darkened after exposure to both solvent vapours, at both exposure times. Secondly, a white crystalline deposit or bloom appeared on the cambium side of one of the four barks (Bark C) exposed to ethanol vapour. This was visible as white specks in one test and a whitish haze in another. The deposits are crystalline and were identified as betulin and lupeol in approximately a 2:1 ratio. They can be removed with swabs moistened with water. No visible white deposits appeared on any of the methanol vapour-exposed samples tested; however, Gilberg reported the possible occurrence of a white surface bloom from exposure to methanol vapour.[2] Given the compositional similarities of the bark extractives obtained using either methanol or ethanol in this study, it seems possible that methanol vapours could also cause the migration of triterpenes to the bark surface. Gilberg also found that covering the bark surface with a sheet of Melinex during airing out to decrease the solvent evaporation rate minimized the appearance of a white bloom due to methanol or ethanol vapour.[2] Other risks of solvent vapour treatment, such as delamination, were not assessed in this research.

Colour measurements following cleaning tests on vapour-exposed barks showed that in most cases, cleaning using water applied by lightly dampened swabs did not alter the colour changes caused by vapour exposure, nor did it itself cause colour change. In only one case was the colour change due to ethanol vapour exposure lessened slightly after swab cleaning, perhaps due to removal of a very thin, visually imperceptible surface deposit.

These colour change results, combined with previous CCI studies on reshaping,[2,5] should assist practitioners in assessing some of the risks and/or benefits of birch bark reshaping treatments using methanol or ethanol vapour. Other exposure conditions should be tested in view of finding a "sweet spot" where a bark absorbs enough solvent vapour to allow for reshaping, but not so much that is causes noticeable colour changes. Controlling birch bark vapour treatments in view of optimizing reshaping and limiting colour change involves controlling exposure time while periodically monitoring both the softening achieved and the weight gain during vapour exposure.

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MATERIALS

Methanol and *Ethanol*: Fisher Scientific, 112 Colonnade Road, Ottawa, Ontario K2E 7L6, Canada; Tel.: 1-800-234-7437; Website: <https://www.fishersci.ca>

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